The Dynamics of Zeroth-Order Ultrasensitivity: A Critical Phenomenon in Cell Biology

Qingdao Huang*

College of Mathematics, Jilin University
Changchun 130012, PRC

and

Hong Qian†

Department of Applied Mathematics, University of Washington
Seattle, WA 98195, USA

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Abstract

It is well known since the pioneering work of Goldbeter and Koshland [Proc. Natl. Acad. Sci. USA, vol. 78, pp. 6840-6844 (1981)] that cellular phosphorylation- dephosphorylation cycle (PdPC), catalyzed by kinase and phosphatase under saturated condition with zeroth order enzyme kinetics, exhibits ultrasensitivity, sharp transition. We analyse the dynamics aspects of the zeroth order PdPC kinetics and show a critical slowdown akin to the phase transition in condensed matter physics. We demonstrate that an extremely simple, though somewhat mathematically "singular" model is a faithful representation of the ultrasentivity phenomenon. The simplified mathematical model will be valuable, as a component, in developing complex cellular signaling netowrk theory as well as having a pedagogic value.

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1 Introduction

Cellular informations are stored in the genome; cellular functions, on the other hand, are carried out by biochemistry. The communications within and between cells are based on biochemical reactions involves enzyme molecules [12]. In particular, reversible chemical modification of enzymes, via phosphorylation- dephosphorylation cycle (PdPC), is one of the most widely used mechanisms in cellular signaling and regulation [4]. In its simplest, canonical form, an enzyme E can be phosphorylated to become E^*, and then dephosphorylated to be back to E. These two reactions are catalyzed by their respective enzymes called kinase and phosphatase. Biologically, the E form of

*huangqd@jlu.edu.cn
†qian@amath.washington.edu

the enzyme is inactive while the E^* form is active. Hence, a change in the concentation of the kinase (or of the phosphatase) leads to a change in the activity of the enzyme [14, 1].

Mathematical modeling of the PdPC kinetics began in the late 1970s [16]. In 1981, Goldbeter and Koshland discovered a surprising result: If both kinase and phosphatase, acting as enzymes for their respective reactions, are operating under a saturated condition, then the change of $E \to E^*$ can be extreme sharp in response to a change in the kinase concentration. They called this phenomenon zeroth-order ultrasensitivity [6]. Before that work, biochemists had believed a sharp, sigmoidal transition is the signature of only enzyme systems with multiple subunits or monomer with multiple binding sites; sigmoidal shaped transition is associated with equilibrium cooperativity. What had not been fully appreciated until recent years is that the PdPC is not a closed equilibrium system, but in fact an open-chemical system [14].

There is now a sizable literature on ultrasensitive PdPC and related biochemical reactions. See [18, 8] for two very recent reports. But all the studies, as far as we know, have been limited to the steady state analysis, with the exception of [19]. The present paper reports a study of the time-dependent behavior of ultrasensitive PdPC. We discover that in addition to the sharp transition, the kinetic of ultrasensitive PdPC exhibit a critical slowdown, i.e., the time to reach steady state tends to infinit when the system approaches the the critical transition point. In a separated study, we have also discovered that at the critical point of transition, the ultrasensitive PdPC exhibits large fluctuations [15, 13].

Putting all these together, this is a reminiscent of the critical phenonemon in physics [3, 17]. The classical phase transition problems in condensed matter physics, however, are equilibrium phenomena. The ultrasensitivity discussed in the present work is a nonequilibrium phenomenon. PdPC is a driven chemical reaction system [14].

2 Phosphorylation-Dephosphorylation Cycle Kinetics

The canonical kinetic system for the PdPC is [6, 9, 7]

$$E + K \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} EK \xrightarrow{k_{12}} E^* + K, \quad E^* + P \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} E^*P \xrightarrow{k_{22}} E + P, \tag{1}$$

in which K and P represent kinase and phosphatase. We follow the Michaelis-Menten approach to model the enzyme reactions [10, 1]. If the amount of kinase is significantlly smaller than that of E, and the amount of phosphatase is significantly smaller than that of E^*, then according to the Michaelis-Menten theory of enzyme kinetics, we have [10, 1]

$$\frac{dc_{E^*}}{d\tau} = \frac{V_1 c_E}{K_{M1} + c_E} - \frac{V_2 c_{E^*}}{K_{M2} + c_{E^*}}, \tag{2}$$

in which the total concentration $c_E + c_{E^*} = c_T$ is a constant. Here c_E and c_{E^*} are the concentrations of E and E^* respectively. The parameters in Eq. (2) are related to the rate constants in (1):

$$K_{Mi} = \frac{k_{-i1} + k_{i2}}{k_{i1}}, \ \ (i = 1, 2); \ \ V_1 = k_{12}[K]_{tot}, \ \ V_2 = k_{22}[P]_{tot}, \tag{3}$$

in which $[K]_{tot}$ and $[P]_{tot}$ are the total concentrations of kinase and phosphatase, respectively.

Equation (2) can be rigorously justified via singular perturbation theory [10, 1]. It is valid for all times, as long as one can neglects the extremely fast kinetics. Eq. (2) in fact is exact for finding

the steady state of the reaction system in (1). The approximation comes from $c_E + c_{E^*} = c_T$ since it neglects the concentrations of EK and E^*P.

We introduce nondimensionalized variables

$$x = \frac{c_{E^*}}{c_T}, \;\; t = \frac{V_2}{c_T}\tau, \tag{4}$$

then we have

$$\frac{dx}{dt} = \frac{\theta(1-x)}{K_1 + 1 - x} - \frac{x}{K_2 + x}, \tag{5}$$

where

$$\theta = \frac{V_1}{V_2} = \frac{k_{12}[K]_{tot}}{k_{22}[P]_{tot}}, \;\; K_1 = \frac{K_{M1}}{c_T}, \;\; K_2 = \frac{K_{M2}}{c_T}. \tag{6}$$

Eq. (5) is the starting point of our current study. The steady state x^* as a function of θ and Ks is well understood [6, 1]. We investigate its time-dependent behavior.

3 A Simple Mathematical Model for Ultrasensitivity

The two terms on the right-hand-side of Eq. (5) represent the *Michaelis-Menten rate law* of enzyme catalyzed reactions, for the kinase and the phosphatase, respectively. Taking the term $r = x/(K_2 + x)$ as example, it has the celebrated "hyperbolic" saturation form, also known as double reciprocal since $1/r$ is a linear function of $1/x$ [1]. Therefore, the rate r as a function of the substrate concentration x is linear, i.e., first-order, when $K_2 \gg x$, but zeroth order when $K_2 \ll x$. When an enzyme is operating under zeroth order condition, the catalyzed reaction rate is independent of the substrate concentration.

The important discovery of Goldbeter and Koshland [6] is that when both kinase and phosphatase in (1) are operating under zeroth order condition, the steady state concentration of phosphorylated enzyme, i.e., x has a very sharp response to the θ, the activation parameter.

One can solve the steady state of Eq. (5), as a root of a quadratic equation, then let K_1 and $K_2 \to 0$. This is the standard method of attack. However, if one lets $K_1 = K_2 = 0$, one immediately obtains

$$\frac{\theta(1-x)}{1-x} = \frac{x}{x}. \tag{7}$$

Being mathematically careful with Eq. (7), one has

$$x = 0; \;\; \text{or} \;\; x = 1; \;\; \text{or} \;\; \theta = 1, \;\; \text{if } x \neq 0, x \neq 1. \tag{8}$$

Noting that x is a monotonic increasing function of θ in Eq. (5), we have steady state x as a function of θ given in Fig. 1A.

This way of setting $K_1 = K_2 = 0$ is in fact the spirit of *singular perturbation* method [10]. Following the same approach and treating $K_1 = K_2 = \epsilon$ as a small parameter, we have the unperturbed equation

$$\frac{dx}{dt} = \theta - 1, \quad (0 < x < 1) \tag{9}$$

The time-dependent solution to the equation is

$$x(t) = (\theta - 1)t + x(0), \quad \text{for} \;\; 0 \leq x \leq 1. \tag{10}$$

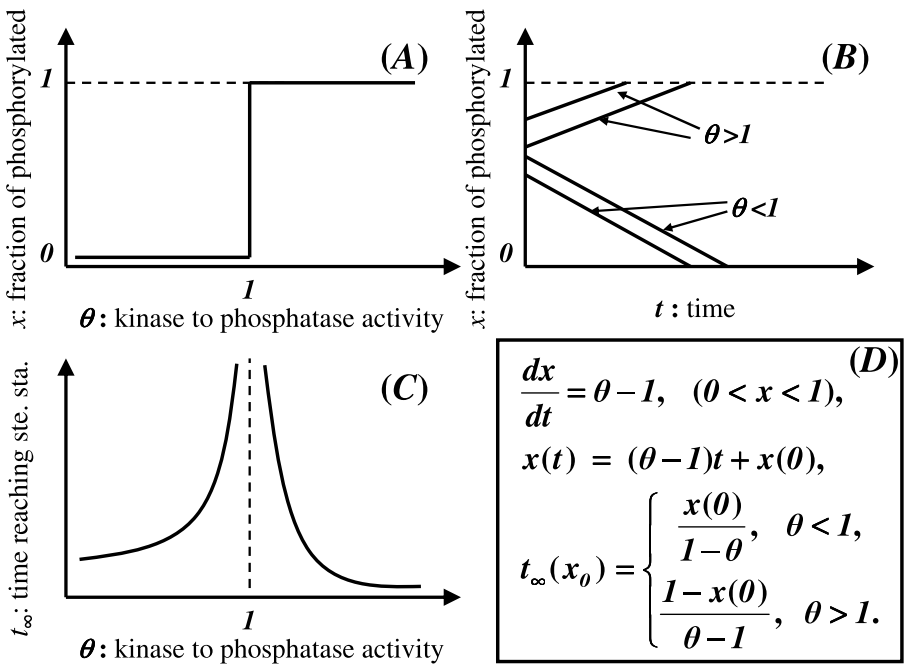

Figure 1: The simple zeroth-order, ultrasensitivity transition in a nutshell: x represents in the fraction of enzyme being phosphorylated; and θ is the ratio of the activities of the kinase and the phosphatase. (A) The activation curve, steady state x as a function of θ, shows a sharp transition. (B) The time-dependent $x(t)$ is a linear function of time t, not exponential as usually expected from first-order chemical kinetics. It reaches steady state in finite time t_∞. (C) The time to reach the steady state, t_∞, exhibit a critical slowdown near the midpoint of the transition, when $\theta = 1$. (D) summarizes the key mathematical results associated with Eq. 9.

This is shown in Fig. 1B. The most interesting insight from this simple solution is that the time reaching the steady state, t_∞, is finite when $\theta \neq 1$. Furthermore, the time

$$t_\infty = \begin{cases} \frac{x(0)}{1-\theta} & \theta < 1, \\ \frac{1-x(0)}{\theta-1} & \theta > 1. \end{cases} \tag{11}$$

This is shown in Fig. 1C.

The $\theta = 1$ is known as the mid-point of transition from unphosphorylated E to the phosphorylated E^*. It is the "critical point" of the transition. A shape transition like in Fig. 1A is usually identified as cooperative phase transition in condensed matter physics. The result on the time in Eq. (11) and Fig. 1C further collaborate this analogue: One of the characteristics of phase transition is the "critical slowdown" in its dynamics [17]. In the theory of phase transition, critical slowdown and shape transition are also intimately related to large fluctuations [17]. This last aspect of PdPC has been discussed in [13, 15].

The easiness and transparency of Eq. (9) makes it very attractive as a simple model for ul-

trasensitivity. The remaining of the paper is to provide a more rigorous justification of the results obtained from Eq. (9), summarized in Fig. 1D, in terms of a perturbation expansion approach. More specifically, we shall try to provide perturbation corrections to the results in Figs. 1B and 1C.

4 Exact Solution to PdPC Kinetics Eq. (5)

To justify the above very simple model for ultrasensitivity, one can in fact solve exactly the nonlinear differential equation in (5). For simplicity, we shall assume $K_1 = K_2 = K$. The Eq. (5) can be re-written as

$$\frac{dx}{dt} = \frac{(1-\theta)x^2 + (\theta - \theta K - K - 1)x + \theta K}{(K+1-x)(K+x)}, \tag{12}$$

the numerator of whose right-hand-side has two roots x_1 and x_2:

$$x_{1,2} = \frac{-\theta + \theta K + K + 1 \pm \sqrt{(1+2K)(\theta-1)^2 + K^2(\theta+1)^2}}{2(1-\theta)}. \tag{13}$$

One of the x_1 and x_2 is $\in [0,1]$, and it is the steady state of the Eq. (5). Let us denote it by x^*, then the other one is $x' = \theta K/[x^*(1-\theta)]$. If $0 \le \theta \le 1$, $x_2 \in [0,1]$, and it is the steady state of the Eq. (5). We also see that $K \to 0, x_2 \to 0$. If $\theta \ge 1$, $x_1 \in [0,1]$, and we have $K \to 0, x_1 \to 1$.

Applying separation of variables and the method of partial fraction to Eq. (12), we have the solution to the Eq. (5)

$$t = \frac{1}{1-\theta}\left\{ x(0) - x(t) + \frac{K}{1-\theta}\ln\left[\left(\frac{x(t)-x^*}{x(0)-x^*}\right)^A \left(\frac{x(t)-x'}{x(0)-x'}\right)^B \right] \right\}. \tag{14}$$

in which,

$$A = \frac{1 + K - \theta K - (1+\theta)x^*}{x^* - x'},$$

$$B = -\frac{1 + K - \theta K - (1+\theta)x'}{x^* - x'}.$$

From Eq. (14), it is immediately clear that if $K = 0$, then $x = (\theta - 1)t + x(0)$. This is the result in Eq. (10). Furthermore, we can compute the "time to steady state". If $K \to 0$, $t \to (x_0 - x(t))/(1-\theta)$. Hence for $x(t_\infty) = 0$, $t_\infty = \frac{x(0)}{1-\theta}$, and for $x(t_\infty) = 1$, $t_\infty = \frac{x(0)-1}{1-\theta}$. These are the results in Eq. (11).

For $K \neq 0$, mathematically exponential decay takes infinite time to actually reach the steady state. To give a measure of the time, however, we consider the time from $x(0) = x^* + 0.01$ to $x(t_\infty) = x^* + 0.001$, for $0 \le \theta < 1$. Similarly, for $\theta > 1$, we consider the time from $x(0) = x^* - 0.01$ to $x(t_\infty) = x^* - 0.001$.

Fig. 2 shows the t_∞ as a function of θ, with various K values. It is shown that with sufficiently small K, the result in Eq. (11) is indeed valid; There is a critical slowdown at the $\theta = 1$ for zeroth order ultrasensitivity with small K.

We can also consider the time-dependent solution given in Eq. (14), $x(t)$. Fig. 1B shows simple linear functions of time, each of which reaches steady state in finite time. To better understand this observation, Fig. 3 shows that the $x(t)$ asymptotically approaches to Eq. (10) with diminishing K.

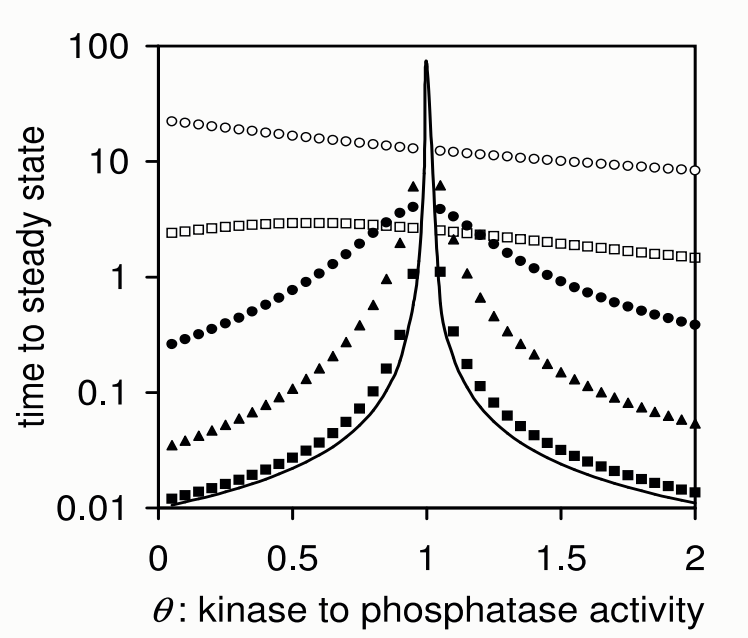

Figure 2: Time to reach steady state (x^*), defined as from initial value $x(0) = x^* \pm 0.01$ to $x(t_\infty) = x^* \pm 0.001$. $\pm$ corresponds to $\theta < 1$ and > 1, respectively. The values for K are 10 (open circles), 1 (open squares), 0.1 (filled circles), 0.01 (filled triangle), and 0.001 (filled square). The solid line is from Eq. (11).

5 Discussion

In developing a mathematical model for a scientific problem, one should always follow Occam's Razor which states, in Albert Einstein's words "as simple as possible, but not simpler." The present paper contains two results: First, it proposes an extremely simple equation for the zeroth-order PdPC, and provides a mathematical proof that the simple model captures all the essential features of the well-accepted, but more complex model. Second, we analyzed the time-dependent behavior of zeroth-order ultrasensitivity and demonstrated a critical slowdown at the transition point; Thus it is a critical phenomenon.

Critical phenomena are the emergent properties of cooperative processes [3, 11, 17]. Calling the zeroth-order ultrasensitivity a critical phenomenon, thus, begs an answer to the question "what is the cooperativity in ultrasensitivity"? Answer to this deeper question is outside the scope of the present paper; we refer the readers to two recent papers on the so-called *temporal cooperativity* [15, 5]. As a matter of fact, a nonequilibrium critical phenomenon in a driven system emerging from a cooperativity in *time* seems to be a novel concept offered by the present mathematical model of the biological system.

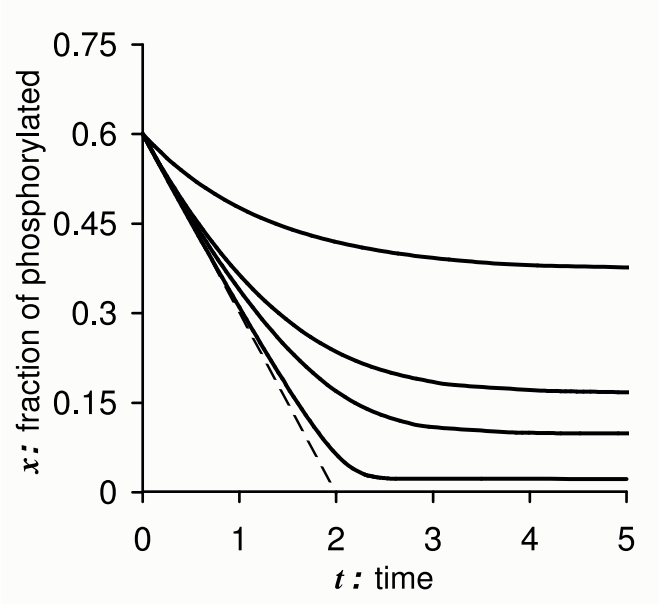

Figure 3: Time dependence of PdPC kinetics. x represents the fraction of substrate enzyme being phosphorylated (c_{E^*}/c_T). All curves have initial value $x(0) = 0.6$ and $\theta = 0.7$. Different curves correspond to different K values: solid lines from top to bottom correspond to $K = 1$, 0.1, 0.05, and 0.01, respectively. The dashed line is from Eq. (10). With diminishing K, the curves asymptotically approaches to the dashed line.

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References

[1] Beard, D.A. and Qian, H. (2008) *Chemical Biophysics: Quantitative Analysis of Cellular Systems.* Cambridge Texts Biomed. Engr., Cambridge Univ. Press, London.

[2] Berg, O.G., Paulsson, J. and Ehrenberg, M. (2000) Fluctuations and quality of control in biological cells: Zero-order ultrasensitivity reinvestigated. *Biophys. J.* **79**, 1228–1236.

[3] Domb, C. (1996) *The Critical Point: A Historical Introduction to the Modern Theory of Critical Phenomena.* Taylor & Francis, London.

[4] Fischer, E.H. and Krebs, E.G. (1955) Conversion of phosphorylase b to phosphorylase a in muscle extracts. *J. Biol. Chem.* **216**, 121-133.

[5] (doi:10.1063/1.2948965) Ge H. and Qian M. (2008) Sensitivity amplification in the phosphorylation-dephosphorylation cycle: Nonequilibrium steady states, chemical master equation, and temporal cooperativity. *J. Chem. Phys.* **129**, 015104.

[6] Goldbeter, A. and Koshland, D.E. (1981) An amplified sensitivity arising from covalent modification in biological systems. *Proc. Natl. Acad. Sci. USA* **78**, 6840-6844

[7] Gunawardena, J. (2005) Multisite protein phosphorylation makes a good threshold but can be a poor switch. *Proc. Natl. Acad. Sci. USA* **102**, 14617-14622.

[8] Huang, Q. and Qian, H. (2009) Ultrasensitive dual phosphorylation dephosphorylation cycle kinetics exhibits canonical competition behavior. *Chaos* **19**, 033109.

[9] Markevich, N.I., Hoek, J.B. and Kholodenko, B.N. (2004) Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J. Cell Biol.* **164**, 353–359.

[10] Murray, J.D. (2002) *Mathematical Biology I: An Introduction*, 3rd Ed., Springer-Verlag, New York.

[11] Poland, D. and Scheraga, H.A. (1970) *Theory of Helix-Coil Transitions.* Academic Press, New York.

[12] Phillips, R., Kondev, J. and Theriot, J. (2008) *Physical Biology of the Cell*, Garland Science, New York.

[13] Qian, H. (2003) Thermodynamic and kinetic analysis of sensitivity amplification in biological signal transduction. *Biophys. Chem*. **105**, 585–593.

[14] Qian, H. (2007) Phosphorylation energy hypothesis: open chemical systems and their biological functions. *Ann. Rev. Phys. Chem*. **58**, 113–142.

[15] Qian, H. and Cooper, J.A. (2008) Temporal cooperativity and sensitivity amplification in biological signal transduction. *Biochem.* **47**, 2211–2220.

[16] Stadtman, E.R. and Chock, P.B. (1977) Superiority of interconvertible enzyme cascades in metabolic regulation: Analysis of monocyclic systems. *Proc. Natl. Acad. Sci. USA* **74**, 2761-2765.

[17] Stanley, H.E. (1999) Scaling, universality, and renormalization: Three pillars of modern critical phenomena. *Rev. Mod. Phys.* **71**, S358-S366.

[18] Thomson, M. and Gunawardena, J. (2009) Unlimited multistability in multisite phosphorylation systems. *Nature* **460**, 274-277.

[19] Wang, Z.-X., Zhou, B., Wang, Q.M. and Zhang, Z.-Y. (2002) A kinetic approach for the study of protein phosphatase-catalyzed regulation of protein kinase activity. *Biochem.* **41**, 7849-7857.